CHAPTER 7

# Pathogens in Wastewater and Biosolids

## INTRODUCTION

A human pathogen is any virus, microorganism, or substance capable of causing disease (*Stedman's Medical Dictionary,* 1977). By this definition, bacteria, parasites, viruses, microbial substances (endotoxins), fungi and other organisms are pathogens. The two general categories are primary and secondary pathogens. Primary pathogens, such as bacteria, parasites and viruses, can invade and infect healthy humans (Burge and Millner, 1980). Secondary pathogens invade and infect a debilitated or an immunosuppressed individual. Often secondary pathogens, such as fungi, are termed *opportunistic pathogens*, since they infect those who have suffered disease, causing severe debilitation.

Fecal coliform, an indicator organism when present in large numbers, indicates the potential presence of pathogens. Intestinal pathogenic bacteria normally react to environmental conditions in a similar manner, as do coliforms. Thus, fecal coliforms are good indicator organisms.

Yanko (1988) demonstrated a strong correlation between fecal coliform densities and frequency of salmonella detection. The data showed that when the log fecal coliform density was below 3 (1000 MPN/g total solids), the frequency of detection of salmonellae was in the range of 0 to 3 MPN/g total solids. Yanko sampled biosolids compost and did not find salmonella in 86 measurements for which the fecal coliform densities were less than 1,000 MPN per gram. This was the basis for the pathogen regulations for Class A biosolids (Farrell, 1992).

One of the greatest concerns with land application of biosolids is the presence of pathogens, for the following reasons:

- Uptake by plants and entry into the food chain
- Movement through the soil and contamination of groundwater with potential contamination of drinking water
- Runoff and erosion containing pathogens and contaminating surface water. This could result in direct exposure to persons contacting the contaminated water (i.e., bathers) or through contamination of drinking water supplies.

©2003 CRC Press LLC

The mere presence of a pathogen is not indicative of the potential for infection or disease. In addition to the presence of organisms, it is important to know how many organisms will cause an infection. This is called the infective dose or dose–response relationship.

Wastewater contains pathogens from human and animal wastes discharged into the sewer system. In addition, surface runoff combined with the sewer system will contain mammalian (especially animal) and avian pathogens. Global and regional conditions such as climate can also affect the type and numbers of certain pathogens. The mobility of our society, ease of travel and influx of individuals from developing countries, especially from semitropical or tropical regions, increase the likelihood of both numbers and types of parasites into wastewaters. The recent increase and appearance of several human and animal organisms or toxins such as *E. coli* 0157:H7, HIV, *Helicobacter pylori* and mad cow (bovine spongiform encephalopathy or BSE) disease could result in these organisms or toxins appearing in wastewaters and biosolids. Little is known of the effect of the wastewater treatment process on these.

*E. coli* 0157:H7 can produce verotoxins causing hemorrhagic colitis (diarrhea that becomes profuse and bloody), hemolytic uremic symptom (bloody diarrhea followed by renal failure) and thrombocytopenic purpura, with symptoms similar to those of hemolytic uremia that also involve the central nervous system (Pell, 1997). Outbreaks from contaminated food and water have been reported (Besser et al., 1993; Wang et al., 1996). Little data exist on virulent strains (e.g., *E. coli* 0157:H7) in biosolids.

Human immunodeficiency virus (HIV) consists of a nucleic acid core, or genome, surrounded by a shell of proteins termed *capsid*. The capsid consists of a bilipid layer, an exterior glycoprotein and a transmembrane glycoprotein. Johnson et al. (1994) discussed the implication of HIV to the wastewater industry. Their main concern was the health implication to workers. As they reported, the discharge of fluids containing the virus would be in small volumes compared to the total discharge of influents, resulting in dilution. Furthermore, the concentration of HIV in human body fluids is low in comparison to other pathogens. Once outside the human body, viable HIV concentrations decline at a first-order rate. Also, the organism cannot survive or reproduce without a host cell.

Once HIV leaves the protective environment of the host cell, it is most susceptible to deactivation and cannot reproduce. Danger to workers would be greater from handling contaminated objects such as condoms or blood-stained cotton gauzes, bandages, or sanitary napkins. The use of protective clothing is recommended.

Several authors studied the survival of HIV in wastewater (Casson et al., 1992, 1997; Enriquez et al., 1993; Slade et al., 1989). The data indicate that HIV survival in wastewater is less than 50 hours. Thus, the danger to the public from the use of biosolids is probably nonexistent. Furthermore, research with enteroviruses and polioviruses has shown that viruses tend to be adsorbed on the organic fraction and deactivated (Johnson et al., 1994). Workers nevertheless need to take precautions.

*Helicobacter pylori* is a human gastrointestinal pathogen involving gastritis, duodenal ulcers and gastric neoplasm (Gilbert et al., 1995). A major cause of peptic ulcer disease and gastric neoplasia, the common pathogen infiltrates about 60% of

©2003 CRC Press LLC

the world's population (Cave, 1997). The mode of entry to the stomach is through the mouth. Infection appears to occur mostly during childhood. Fecal oral spread is a possibility, though fecal excretion has not been demonstrated (Cave, 1997). It has been very difficult to demonstrate its presence in the environment and is not presently recovered from sewage. Cave reported that changes in sanitary conditions since World War II resulted in a substantial decrease of the organism. Grubel et al. (1997) suggested that flies may pick up *H. pylori* in human wastes, particularly from untreated sewage, and deposit contaminated fly excreta on food or even directly onto the oral mucous membranes of young children.

"Mad cow" disease is not the result of a living pathogen. This disease in humans is also referred to as Creutzfeld-Jacob. The manifestation is spongy holes in the brain that is believed to be caused by prions, which are proteins that sit on the surface of brain cells. The deadly agent is a misfolded or misshapen prion. It is believed that when an abnormal prion is ingested from food, it travels to the brain, where in some way it subverts or changes the normal prion protein into an abnormal shape. Even if contaminated food is discharged into the wastewater stream, these proteins will likely be degraded during secondary treatment. Furthermore, in soils the proteins would be a source of organic nitrogen and transformed to inorganic nitrogen. Their large molecular structure would preclude any uptake by plants.

The primary pathogens found in wastewater and biosolids can be grouped into four major categories:

- Bacteria
- Enteric viruses
- Protozoa
- Helminths
  - Nematodes (round worms)
  - Cestodes (tapeworms)

Examples of secondary pathogens in biosolids include:

- *Escherichia coli* (*E. coli*)
- *Klebsiella* sp.
- *Yersinia* sp.
- *Aspergillus fumigatus*
- *Listeria*

Although *E. coli* is often termed a secondary pathogen, pathogenic strains of *E. coli* can cause diarrhea and gastroenteritis (Sack, 1975). Fatalities have occurred in children. A recent outbreak in Japan infected 8000 children, resulting in several deaths.

Endotoxins and organic dust are examples of pathogenic substances that may be in biosolids or biosolid-derived products. These and other organisms can be airborne or aerosolized during land application, composting, or heat drying (Sorber et al., 1984).

On February 19, 1993, USEPA promulgated regulations for the utilization and disposal of biosolids. These regulations, titled "Standards for the Use or Disposal

©2003 CRC Press LLC

**Table 7.1a Some Bacteria Found in Wastewater, Sludge and Biosolids and the Diseases They Transmit**

| Bacteria | Disease |
|---|---|
| *Salmonella* spp. (approximately 1700 types) | Salmonellosis<br>Gastroenteritis |
| *Salmonella typhi* | Typhoid fever |
| *Mycobacterium tuberculosis* | Tuberculosis |
| Shigellae (4 species) | Shigellosis<br>Bacterial dysentery<br>Gastroenteritis |
| *Escherichia coli (*pathogenic strains) | Gastroenteritis |
| *Yersinia* spp. | Yersinosis |
| *Campylobacter jejuni* | Gastroenteritis |
| *Vibrio cholerae* | Cholera |

*Data sources*: Epstein and Donovan, 1992; Akin et al., 1983; Ward et al., 1984; Smith and Farrell, 1996.

of Sewage Sludge; Final Rules 40 CFR Part 503," were published in the *Federal Register* Volume 38, Number 32. The rule referred to as Part 503 governs land application of biosolids, including distribution and marketing of biosolid products. The intent of the rule was to encourage beneficial use of biosolids while protecting human health and the environment.

Pathogen and vector attraction reduction (VAR) are discussed under Subpart D, Section 503.30. Two requirements of sewage sludge with respect to pathogens must be met and one of the VAR requirements must be met. Chapter 11 discusses the federal regulations as well as state and several other country regulations. Part 503 regulations do not regulate bioaerosols or secondary pathogens.

This chapter provides information on primary and secondary pathogens in biosolids and other domestic wastes; exposure, infectivity and risk; effect of wastewater treatment on removal of pathogens; and effect of biosolids treatment on destruction of pathogens. Survival in soils and plants is covered in Chapter 8.

## PATHOGENS IN WASTEWATER, SLUDGE, AND BIOSOLIDS

The objective of wastewater treatment is to remove pathogens and disinfect effluent prior to discharge into water courses. The efficiency of removal varies with the different unit processes. It also depends on the organisms and their physical and biological properties. For example, many parasites survive the wastewater treatment process and accumulate in the solids fraction, termed sludge, as a result of their densities. Parasitic eggs tend to settle out in sludge at a more rapid rate than protozoan cysts (Farrell et al., 1996).

Numerous pathogens are found in wastewater and sludge (see Tables 7.1a, b, c and d). The pathogenic bacteria of major concern are *E. coli* (pathogenic strains), *Salmonella* sp., *Shigella* sp. and *Vibrio cholerae* (Kowal, 1985).

The type and densities of pathogens in biosolids are primarily a function of the wastewater and biosolids treatment processes. Pedersen et al. (1981) found that the

©2003 CRC Press LLC

**Table 7.1b Some Viruses Found in Wastewater, Sludge and Biosolids and the Diseases They Transmit**

| Virus | Disease |
|---|---|
| Adenovirus (31 types) | Conjunctivitis, respiratory infections, gastroenteritis |
| Polio virus | Poliomyelitis |
| Coxsackievirus | Aseptic meningitis, gastroenteritis |
| Echovirus | Aseptic meningitis |
| Reovirus | Respiratory infections, gastroenteritis |
| Norwalk agents | Epidemic gastroenteritis |
| Hepatitis viruses | Infectious hepatitis |
| Rotaviruses | Gastroenteritis, infant diarrhea |

*Data sources*: Epstein and Donovan, 1992; Ward et al., 1984; Smith and Farrell, 1996.

primary way to reduce pathogenic organisms is by removing their food sources. The majority of the data on pathogens in biosolids, as a result of the wastewater treatment, has been generated prior to 1980.

Sekla et al. (1980) isolated 54 strains of salmonella from 38 samples of sludge and 16 samples of effluent, representing 13 serotypes. Theis et al. (1978) reported that positive samples of helminth were recovered from sludge from Los Angeles, Sacramento and Oakland, California; as well as Springfield, Missouri; Hopkinsville, Kentucky and Frankfort, Indiana.

Koenraad et al. (1997) found that the numbers of *Campylobacter* in wastewater in the United Kingdom, Germany, Italy and the Netherlands ranged from 50 to more than 50,000 MPN/100 ml. Ten species are known to infect humans, resulting in enteritis, fever, gingivitis, periodontitis and diarrhea. Cliver (1975) recovered human intestinal viruses from waste and return-activated sludge. The enteroviruses included poliovirus and reovirus.

Wellings et al. (1976) isolated Echo-7 virus from biosolids after 13 days on biosolid-drying beds. Moore et al. (1978) showed that 89% to 99% of the viruses were associated with solids from activated sludge aeration basins. In four cities that were studied, enteroviruses were detected in the range of 190 to 950 PFU/l. Grabow (1968) and Foster and Engelbrecht (1973) reported that more than 100 distinct serotypes of viruses are present in wastewater. Their data are summarized in Table 7.2.

Individuals exposed to a pathogenic organism may not necessarily become infected. The dose–response relationship is an indication of the infective dose. This dose–response is difficult to assess since tolerance for individuals varies widely (Jones et al., 1983). Furthermore, infection does not necessarily result in a disease. Table 7.3 shows dose–response for several pathogens (Bryan, 1977).

Akin (1983) reviewed the literature on infective dose data for enteroviruses and other pathogens. The widest dose response range occurred with enteric bacteria. *Salmonella* spp. required $10^5$ to $10^8$ cells to produce a 50% disease rate in healthy adults. Three species of *Shigella* produced illness in subjects administered 10 to 100 organisms. Administering small doses, 1 to 10, cysts of *Entamoeba coli* and *Giardia lamblia* caused amoebic infections. Very low doses of enteric viruses were found to produce infection. Hornick et al. (1970) administered various doses of *Salmonella*

©2003 CRC Press LLC

**Table 7.1c Some Protozoa and Helminth Parasites Found in Wastewater, Sludge and Biosolids and the Diseases They Transmit**

| Organism | Disease |
|---|---|
| **Protozoa** | |
| *Entamoeba histolytica* | Amoebic dysentery, amebiasis |
| *Giardia lamblia* | Giardiasis |
| *Balantidium coli* | Balantidiasis |
| *Naegleria fowleri* | Meningoencephalitis |
| *Cryptosporidium* spp. | Gastroenteritis |
| *Toxoplasma gondii* | Toxoplasmosis |
| **Helminths – Nematodes** | |
| *Ascaris lumbricoides* | Ascariasis |
| *Ascaris suum* | Respiratory |
| *Ancylostoma duodenale* | Hook worm, ancylostomiasis |
| *Necator americanus* | Hookworm |
| *Ancylostoma braziliense* (cat hookworm) | Cutaneous larva migrans |
| *Ancylostoma caninum* (dog hookworm) | Cutaneous larva migrans |
| *Enterobius vermicularis* (pinworm) | Enterobiasis |
| *Strongyloides stercoarlis* (threadworm) | Strongyloidiasis |
| *Toxocara cati* (cat roundworm) | Visceral larva migrans |
| *Toxocara canis* (dog roundworm) | Visceral larva migrans |
| *Trichuris trichiura* (whip worm) | Trichuriasis |
| **Helminths – Cestodes** | |
| *Taenia saginata* (Beef tapeworm) | Taeniasis |
| *Taenia solium* (pork tapeworm) | Taeniasis |
| *Necator americanus* | Hookworm disease |
| *Hymenolepis nana* (dwarf tapeworm) | Taeniasis |
| *Echinococcus granulosus* (dog tapeworm) | Unilocular echinococcosis |
| *Echinococcus multilocularis* | Alveolar hydatid disease |

*Data sources*: Akin et al., 1983; Epstein and Donovan, 1992; Smith and Farrell, 1996.

*typhi* to 14 adult volunteers and found that none showed any symptoms when 1000 organisms were administered. When a dose of 100,000 organisms was administered, 28% of the adults became ill; 95% of the subjects were ill when 1,000,000,000 organisms were administered.

©2003 CRC Press LLC

**Table 7.1d Pathogenic Fungi that May be Present in Sludge and Biosolids**

| Fungi | Disease |
|---|---|
| *Aspergillus fumigatus* | Respiratory infections |
| *Candida ablicans* | Candidiasis |
| *Cryptococcus neoformans* | Subacute chronic meningitis |
| *Epidermophyton* spp. and *Trichophyton* spp. | Ringworm and athlete's foot |
| *Trichosporon* spp. | Infection of hair follicles |
| *Phialophora* spp. | Deep tissue infections |

*Source:* Adapted from Fradkin, 1989.

**Table 7.2 Viruses in Wastewater and Sewage Sludge**

| Virus | Disease |
|---|---|
| Hepatitis A virus | Infectious hepatitis |
| Norwalk and Norwalk-like viruses | Gastroenteritis |
| Rotaviruses | Gastroenteritis |
| Enteroviruses | |
| Poliovirus | Poliomyelitis |
| Coxsackieviruses | Meningitis, pneumonia, hepatitis, cold-like symptoms |
| Echoviruses | Meningitis, encephalitis,cold-like symptoms |
| Reovirus | Respiratory infections, gastroenteritis |
| Astroviruses | Gastroenteritis |
| Caliciviruses | Gastroenteritis |

*Source:* USEPA, 1999.

**Table 7.3 Dose-Response for Several Pathogens**

| Pathogen | Approximate Dose to Produce Disease in 25-75% of Subjects Tested | Minimum Dosage to Produce Disease in Any Individual Number of Organisms |
|---|---|---|
| *Shigella* sp. | $10^2$–$10^5$ | $10^1$ |
| *Salmonella* sp. | $10^5$–$10^9$ | $10^4$ |
| *Escherichia coli* | $10^6$–$10^{10}$ | $10^6$ |
| *Vibrio cholerae* | $10^3$–$10^{11}$ | $10^3$ |
| *Streptococcus faecalis* | $>10^{10}$ | $10^{10}$ |
| *Entamoeba coli*[1] | $10^1$–$10^3$ | $10^1$ |
| *Giardia lamblia*[1] | – | $10^1$ |

[1] The dosage caused infection and not the disease.

*Source*: Adapted from Bryan, 1977.

Pharen (1987) reviewed the literature on infective doses for bacteria and viruses. In addition to the infective dose, other factors, such as age and general health, are important. Pharen states, "However, people do not live in a germ- nor risk-free society. Microorganisms are present almost everywhere — in the air, the soil and on objects that people touch." Additional information on the infective dose data as reported in the literature is shown in Table 7.4.

©2003 CRC Press LLC

**Table 7.4 Reported Infective Dose for Several Organisms**

| Organism | Infective Dose | Range | Reference |
|---|---|---|---|
| | | **Bacteria** | |
| *Clostridium perfringens* | $10^6$ | $10^6$–$10^{10}$ | Kowal, 1985 |
| *Escherichia coli* | $10^4$ | $10^4$–$10^{10}$ | Keswick, 1984; Kowal, 1985 |
| *Salmonella* (various species) | $10^2$ | $10^2$–$10^{10}$ | Kowal, 1985; |
| *Shigella dysenteriae* | 10–$10^2$ | 10–$10^9$ | Kowal, 1985; Keswick, 1984; Levine et al., 1973 |
| *Shigella flexneri* | $10^2$ | $10^2$–$10^9$ | Kowal, 1985 |
| *Streptococcus faecalis* | $10^9$ | $10^9$–$10^{10}$ | Kowal, 1985 |
| *Vibro cholerae* | $10^3$ | $10^3$–$10^{11}$ | Kowal, 1985; Keswick, 1984 |
| | | **Viruses** | |
| Echovirus 12 | $HID_{50}$[a] 919 PFU[b] HID1[c] 17 PFU estimated | 17–919 PFU | Kowal, 1985 |
| Polio virus | 1 $TCID_{50}$[d] , <1 PFU | 4 ¥ $10^7$ $TCID_{50}$ for infants; 0.2–5.5 ¥ $10^6$ PFU for infants | Kowal, 1985 |
| Rotavirus | $HID_{50}$ 10 ffu $HID_{25}$ 1 ffu estimated | 0.9–9 ¥ $10^4$ | Ward et al., 1986 |
| | | **Parasites** | |
| *Entamoeba coli* | 1–10 cysts | 1–10 cysts | Kowal, 1985 |
| *Cryptosporidium* | 10 cysts 30 oocysts | 10–100 cysts | Casmore, 1991 Dupont et al., 1995 |
| *Giardia lamblia* | 1 cyst estimated | NR | Kowal, 1985 |
| Helminths | 1 egg | NR | Kowal, 1985 |

[a]HID = Human infective dose.
[b] Plaque forming units per gram dry weight.
[c] $TCID_{50}$ = 50% tissue culture infectious dose.
[d] ffu = focus forming units.

## REMOVAL OF PATHOGENS BY WASTEWATER TREATMENT PROCESSES

Several physical, chemical and biological factors inactivate pathogens. Reimers et al. (1996) discuss these factors, which are summarized in Table 7.5.

The type and densities of pathogens in biosolids is primarily a function of the wastewater and biosolids treatment processes. Pedersen et al. (1981) indicate that the primary reduction of pathogenic organisms results through removal of the food sources. The majority of the data on pathogens in biosolids undergoing wastewater

©2003 CRC Press LLC

**Table 7.5 Physical, Chemical and Biological Factors Affecting Inactivation of Pathogens**

| Physical | Chemical | Biological |
|---|---|---|
| Temperature | pH (acids/alkali) | Antagonistic organisms |
| Applied fields | Ozone | Digestion (aerobic/anaerobic) |
| Microwave irradiation | Ammonia | Composting |
| Infrared irradiation | Nitrous acids | Alkaline composting |
| Ultra sonication | Phosphoric acid | |
| Magnetic fields | Nitric acid | |
| Pulsing electrostatic/electrolytics | Alkaline agents | |
| Desiccation | Sulfuric acid | |

*Source*: Reimers et al., 1996, pp. 51–74, *Stabilization and Disinfection — What Are Our Concerns*, Water Environment Federation, Dallas, TX. With permission.

treatment has been generated prior to 1980. Table 7.6 provides some of the early data (Pedersen et al., 1981). Data on viruses were limited due to poor recovery from solids. Although methodologies for the enumeration of pathogens in biosolids have been shown to be deficient, updates in more recent years have been scant (Yanko et al., 1995). Parsons et al. (1975) summarized findings in the literature at that time on the effect of wastewater treatment on pathogen destruction. The authors concluded that wastewater treatments significantly reduced certain pathogenic microorganisms, but no single process yielded an effluent virtually free of pathogenic microorganisms.

During primary and secondary treatment, many pathogens are destroyed. Foster and Engelbrecht (1973) summarized the early data, shown in Table 7.7. Many of the pathogens removed during primary and secondary treatment will be associated with the biosolids. Land application of biosolids requires disinfection and stabilization. Dahab et al. (1996) determined the concentrations of fecal coliform, fecal streptococci and *Salmonella* spp. in primary sludge in nine different wastewater treatment plants. Fecal coliform densities varied from 12 to 61 million MPN/g of total solids (TS), the most probable number per gram of total solids. The average was 36 million MPN/g of TS. Fecal streptococcus densities ranged from a low of 2.6 million to a high of 40 million MPN/g TS. *Salmonella* spp. densities varied from 217 to 1000 MPN/g TS for eight of the treatment plants. At the ninth plant, the levels were 3140 MPN/g of TS.

Stadterman et al. (1995) evaluated the efficiency of the removal of *Cryptosporidium* oocysts by the waste-activated sludge treatment and anaerobic digestion. The authors reported that the total oocyst removal in sewage treatment was 98.6%. After 24 hours 99.9% of the oocysts were eliminated by anaerobic digestion. Koenraad et al. (1997) found that the wastewater treatment processes reduced the levels of *Campylobacter* by several factors, but many of the organisms survived. Anaerobic digestion had little effect on reducing the numbers, but aerobic digestion was effective in eliminating the organism.

Malina (1976) reported an early review of the inactivation of viruses by various wastewater treatment processes. Some of the data is summarized in Table 7.8. The author points out that in many of the studies, the virus titer was far in excess of

©2003 CRC Press LLC

**Table 7.6 Density Levels of Indicator Organisms and Pathogens in Primary, Secondary and Mixed Biosolids[a]**

| Organism | Primary | | Secondary | | Mixed | |
|---|---|---|---|---|---|---|
| Total coliform bacteria | $1.2 \times 10^8$ | Gaby, 1975; Noland et al., 1978 | $7.1 \times 10^8$ | Noland et al., 1978; Bovay Engineers,1975 | $1.1 \times 10^9$ | Berg & Berman, 1980; Laconde et al., 1978a; b |
| Fecal coliform bacteria | $2.0 \times 10^7$ | Gaby, 1975; Noland et al., 1978; Counts & Shuckrow, 1974; SAC, 1979 | $8.3 \times 10^6$ | Noland et al., 1978; Bovay Engineers, 1975; Counts & Shuckrow, 1974 | $1.9 \times 10^5$ | Counts & Shuckrow, 1974; Berg & Berman, 1980; Laconde et al., 1978a; b |
| Fecal streptococci | $8.9 \times 10^5$ | Gaby, 1975; Noland et al., 1978; Counts & Shuckrow, 1974; SAC, 1979 | $1.7 \times 10^6$ | Noland et al., 1978; Bovay Engineers, 1975; Counts & Shuckrow, 1974 | $3.7 \times 10^6$ | Counts & Shuckrow, 1974; Berg & Berman, 1980; Laconde et al., 1978a; b |
| *Salmonella* sp. | $4.1 \times 10^2$ | Noland et al. 1978; Counts & Shuckrow, 1974; SAC, 1979; Moore et al., 1978 | $8.8 \times 10^2$ | Noland et al., 1978; Counts & Shuckrow, 1974 | $2.9 \times 10^2$ | Counts & Shuckrow, 1974; Laconde et al., 1978a, b |
| *Pseudomonas aeruginosa* | $2.8 \times 10^3$ | Noland et al., 1978; Counts & Shuckrow, 1974 | $1.1 \times 10^4$ | Noland et al., 1978; Counts & Shuckrow, 1974 | $3.3 \times 10^3$ | Counts & Shuckrow, 1974 |
| *Ascaris* sp. | $7.2 \times 10^2$ | Reimers et al., 1980 | $1.4 \times 10^3$ | Reimers et al., 1980 | $2.9 \times 10^2$ | Reimers et al., 1980 |
| *Trichuris trichiura* | $1.0 \times 10^1$ | Reimers et al., 1980 | $<1.0 \times 10^1$ | Reimers et al., 1980 | 0 | Reimers et al., 1980 |
| *Trichuris vulpis* | $1.1 \times 10^2$ | Reimers et al., 1980 | $<1.0 \times 10^1$ | Reimers et al., 1980 | $1.4 \times 10^2$ | Reimers et al., 1980 |
| *Toxocara* sp. | $2.4 \times 10^2$ | Reimers et al., 1980 | $2.8 \times 10^2$ | Reimers et al., 1980 | $1.3 \times 10^3$ | Reimers et al., 1980 |
| *Hymenolpepis diminuta* | $6.0 \times 10^0$ | Reimers et al., 1980 | $2.0 \times 10^1$ | Reimers et al., 1980 | 0 | Reimers et al., 1980 |
| Enteric viruses[b] | $3.9 \times 10^2$ | Nath & Johnston, 1979; Moore et al., 1978; Hurst et al., 1978; Nielsen & Lydholm, 1980 | $3.2 \times 10^2$ | Moore et al., 1978; Hurst et al., 1978; Nielsen & Lydholm, 1980 | $3.6 \times 10^{2c}$ | Nielsen & Lydholm, 1980 |

[a]Data are average geometric means of organisms per gram solids dry weight.
[b] Plaque forming units per gram dry weight (PFU/gdw).
[c] $TCID_{50}$ = 50 percent tissue culture infectious dose.

*Source*: Pedersen, 1981.

©2003 CRC Press LLC

**Table 7.7 Pathogen Removal Efficiency during Primary and Secondary Wastewater Treatment**

| Pathogen | Primary Treatment % Removal Efficiency | Secondary Treatment % Removal Efficiency | Trickling Filter % Removal Efficiency |
|---|---|---|---|
| Bacteria[a] | 50–90 | 90–99 | 90–95 |
| *Salmonella* sp.[b] | 15 | 96–99 | 84–99.9 |
| *Mycobacterium* sp.[b] | 48–57 | Slight to 87 | 66–99 |
| Protozoan cysts[a] | 10–50 | 50 | 50–95 |
| Amoebic cysts[b] | No reduction in 3 hours | No apparent removal | 11–99.9 |
| Helminth ova[a] | 72–78 | No apparent removal | 62–76[b]<br>50– 90[a] |
| Virus[ab] | 3 to extensive removal[b]<br>0–30[a] | 76–99[b]<br>90–99[a] | 0–84[b]<br>90–95[a] |

[a] Feachem et al. (1980)
[b] Foster and Engelbrecht (1973)

indigenous levels of 4000 to 7000. However, inactivation would occur also at lower levels. The data show that wastewater treatment is only partially effective in the inactivation of viruses but, chlorine or ozone disinfection is very effective. Removal of organisms during wastewater treatment displaces them from the liquid stream. However, they become associated with the solids.

## EFFECT OF BIOSOLIDS TREATMENT

The solids resulting from wastewater treatment must undergo further treatment prior to land application. Land application of biosolids requires the disinfection and stabilization of biosolids. The objective is to reduce the level of pathogens, reduce vector attraction and produce a stabilized product — that is, a product that would not decompose very rapidly and produce offensive odors. Table 7.9 shows the general effect of wastewater treatment and densities of microorganisms in effluent and biosolids (NRC, 1996).

Temperature is very effective in the destruction of pathogens. The time–temperature relationships for pathogen destruction were used in the USEPA 503 regulations (USEPA,1992). Several biosolid processes rely on temperature to meet Class A biosolids. These include: composting, heat drying, alkaline stabilization and thermal digestion. Table 7.10 shows the thermal destruction of several pathogens and parasites. This data is derived from pathogen destruction in liquids where temperature is much more uniform throughout the mass. With biosolid and biosolid products, a longer period of time is needed to ensure that every particle within the mass is subjected to the temperature.

USEPA in the 503 regulations requires that either a Class A or B biosolid be produced prior to land application. Class A biosolid is a material that has undergone treatments that reduce pathogens to very low or undetectable levels. A less stringent requirement is allowed for Class B. Details of the regulations are provided in Chapter 11.

©2003 CRC Press LLC

**Table 7.8 Effect of Municipal Wastewater Treatment on Viral Inactivation**

| Wastewater Process | Virus | Percent Removal | Titer PFU/l | Reference |
|---|---|---|---|---|
| Primary clarification | Bacteriophage F2 | 37.1 | $(6.7–7.6) \times 10^5$ | Sherman, 1975 |
| | Polio 1 (Mahoney) | 26–55 | $2 \times 10^8$ | Clarke et al., 1964 |
| | Polio 1,2,3 (Sabin) | 0–12 | * | England et al., 1967 |
| Activated biosolids | Bacteriophage T2 | 98 | $(3\text{-}50) \times 10^5$ | Kelly et al., 1961 |
| | Coxsackie A9 | 96–99.4 | $3 \times 10^8$ | Clarke et al., 1961 |
| | Polio 1 (Sabin) | 98 | $7.7 \times 10^4$ | Malina and Melbard, 1974 |
| | Polio 1 (MK 500) | 64–78 | $(2–200) \times 10^6$ | Kelly et al., 1961 |
| | Polio 1 (Mahoney) | 79–94 | $7 \times 10^7$ | Clarke et al., 1961 |
| | Polio 1,2,3 (Sabin) | 76–90 | * | England et al., 1967 |
| Aerated lagoons | Polio 1 (Sabin) | 99 | $1.6 \times 10^3$ | Ranganathan et al., 1974 |
| Oxidation ponds | Polio 1 (attenuated) | 92 | $5.6 \times 10^5$ | Malina et al., 1975 |
| | Polio 1 (Sabin) | 99 | $3.3 \times 10^3$ | Malina and Melbard, 1974 |
| | Reovirus | 95 | 20,000* | Nupem et al., 1974 |
| | Polio 1 (Mahoney) | 99.97-ND | $(6–1800) \times 10^3$ | Malina and Melbard, 1974 |
| Trickling filter | Bacteriophage F2 | 18.9 | $(5.9–7.5) \times 10^5$ | Sherman, 1975 |
| | Coxsackie A9 | 84 | $3 \times 10^9$ | Clarke and Chang, 1975 |
| | Echovirus 12 | 83 | $7 \times 10^9$ | Clarke and Chang, 1975 |
| | Polio 1 | 85 | $4 \times 10^9$ | Clarke and Chang, 1975 |
| Disinfection chlorine | Bacteriophage F2 | 99.997 | No data | Anonymous, 1975 |
| | Polio | 99 | $10^{9}$** | Kott et al., 1975 |
| Disinfection ozone | Bacteriophage F2 | 100 | $1 \times 10^{11}$ | Pavoni and Tittlebaum, 1974 |
| | Cocsackie B3 | 99.9 | $2.5 \times 10^2$ | Keller, 1974 |
| | Polio I | 99.994 | $(1.4–6.3) \times 10^7$ | Majumdar et al., 1974 |
| | Polio II | 99.99 | $5 \times 10^2$ | Keller, 1974 |

*Natural levels following immunization.
**$TCID_{50}$/l = 50% tissue culture infectious dose.

*Source*: Adapted from Malina, 1976.

The processes used to achieve the regulatory requirements are:

- Aerobic digestion
- Anaerobic digestion
- Composting
- Heat drying
- Alkaline stabilization

©2003 CRC Press LLC

**Table 7.9 Effect of Wastewater Treatment on the Densities of Microorganisms in Effluent and Biosolids**

| Organism | Raw Sewage | Number per 100 ml of Effluent | | | Numbers per gram of Biosolids | |
|---|---|---|---|---|---|---|
| | | Primary Treatment | Secondary Treatment | Tertiary[a] Treatment | Raw | Digested[b] |
| Fecal coliform MPN[c] | $1\times10^{9}$ | $1\times10^{7}$ | $1\times10^{6}$ | <2 | $1\times10^{7}$ | $1\times10^{6}$ |
| *Salmonella* MPN | 8,000 | 800 | 8 | <2 | 1,800 | 18 |
| *Shigella* MPN | 1,000 | 100 | 1 | <2 | 220 | 3 |
| Enteric virus PFU[d] | 50,000 | 15,000 | 1,500 | 0.002 | 1,400 | 210 |
| Helminth ova | 800 | 80 | 0.08 | <0.08 | 30 | 10 |
| *Giardia lamblia* cysts | 10,000 | 5,000 | 2,500 | 3 | 140 | 43 |

[a] Includes coagulation, sedimentation, filtration and disinfection.
[b] Mesophilic anaerobic digestion.
[c] MPN = most probable number.
[d] PFU = plaque forming units.
*References*: USEPA, 1991 and 1992; Dean and Smith, 1973; Feachem et al., 1980; Engineering Science, 1987; Gerba, 1983.

**Table 7.10 Thermal Destruction of Several Pathogens and Parasites**

| Organism | Thermal Death Points |
|---|---|
| *Salmonella typhosa* | No growth beyond 46°C; death within 30 min at 55° to 60°C |
| *Salmonella* spp. | Death within 1 h at 56°C; death within 15 to 20 min at 60°C |
| *Shigella* spp. | Death within 1 h |
| *Escherichia coli* | Most die within 1 h at 55°C and within 15 to 20 min at 60°C |
| *Micrococcus pyogenes* var. *aureus* | Death within 10 min at 50°C |
| *Streptococcus pyogenes* | Death within 10 min at 54°C |
| *Microbacterium tuberculosis* var. *hominis* | Death within 15 to 20 min at 66°C |
| *Mycobacterium diptheriae* | Death within 45 min at 55°C |
| *Endamoeba histolytica* | Thermal death is 68°C |
| *Tania saginata* | Death within 5 min at 71°C |
| *Trichinella spiralis* larvae | Thermal death point is 62 to 72°C |
| *Necator americanus* | Death within 50 min at 45°C |

*Source*: NRC, 1996.

©2003 CRC Press LLC

## Aerobic Digestion

Aerobic digestion has been carried out under mesophilic conditions ranging in temperature from ambient to 37°C and retention times of 10 to 20 days. More recently, there has been an evaluation of thermophilic aerobic digestion in order to meet Class A biosolids. Relatively few data exist on the effectiveness of aerobic digestion on pathogen destruction. Novak et al. (1984) studied the survival of indicator organisms during mesophilic aerobic digestion of biosolids at various sludge ages in the oxidation ditch. The data were variable. One-log reduction in Fecal streptococci occurred in 1 to 16 days at 20°C. For a 2-log reduction of the organisms at 20°C, an aeration time ranging from 6 to 40-plus days was required. Kebina and Plosheva (1974), as cited by Fitzgerald and Ashley (1977), found that *Ascaris suum* ova in mesophilic aerobically stabilized biosolids failed to develop in the absence of oxygen. The ova were able to develop after aeration at 20 to 27°C. *Salmonella* sp. and *E.Coli* were reduced in density by 1 log after several days of retention. Farrah et al. (1981) reported that aerobically digested biosolids contained enteric viruses from 14 to 260 $TCID_{50}$/g (50% tissue culture infectious dose).

Autothermal thermophilic aerobic digestion (ATAD) is a relatively new process designed to produce PFRP or Class A Biosolids for land application. Therefore, data on pathogen destruction are limited. Vik and Kirk (1996) reported that since the inception of the first ATAD in 1994, fecal coliform levels measured weekly were less than 244 MPN/g of TS and most of the levels were below 100 MPN/g of TS.

Dahab et al. (1996) determined densities of fecal coliform, fecal streptococcus and *Salmonella* sp. in four wastewater treatment plants. The range of fecal coliform densities ranged from 50,000 to 3.8 million MPN/g of TS with an average density of 1.7 million MPN/g of TS. One of the four plants could not meet the USEPA Class B biosolids criteria. This was believed by the authors to be as a result of relatively low hydraulic retention time and low sludge age. Fecal streptococcus densities ranged from 30,000 to 2.23 MPN/g of TS. The average fecal streptococcus density was 850,000 MPN/g of TS. *Salmonella* sp. varied considerably. Two of the four plants had densities of 80 and 82 MPN/4g of TS and two plants had densities of 2340 and 3840 MPN/4g of TS. *Cryptosporidium* oocysts were destroyed during thermophilic aerobic digestion when temperatures exceeded 55°C (Whitmore and Robertson, 1995).

In many cases, aerobically digested biosolids can meet USEPA Class B biosolids and be land applied. ATAD systems may be able to meet Class A biosolids.

## Anaerobic digestion

Anaerobic digestion can be carried out under mesophilic or thermophilic conditions. Mesophilic anaerobic digestion is usually achieved at temperatures 30°C to 38°C, whereas in thermophilic anaerobic digestion the temperatures range from 50°C to 60°C. Primary or secondary sludge is fed intermittently or continuously into sealed vessels that preclude free oxygen. Although the primary purpose of anaerobic digestion is solids reduction, other benefits are methane production and pathogen reduction.

©2003 CRC Press LLC

**Table 7.11 Density Levels and Reduction of Indicator Bacteria and Pathogenic Microorganisms by High Rate Mesophilic Anaerobic Digestion**

| Organism | Density Level[1] per 100 ml | Log Reduction Mean[2] | Log Reduction Range | Reference |
|---|---|---|---|---|
| Total coliform | $3 \times 10^7$ | 2.05 | 1.78–2.30 | Berg and Berman, 1980; Lue-Hing et al., 1979; Jewell et al., 1980 |
| Fecal coliform | $2 \times 10^6$ | 1.84 | 1.44–2.30 | SAC 1979; Berg and Berman, 1980; Lue-Hing et al., 1979; Jewell et al., 1980 |
| Fecal streptococcus | $9 \times 10^5$ | 1.48 | 1.10–1.94 | SAC, 1979; Berg and Berman, 1980; Lue-Hing et al., 1979; Jewell et al., 1980 |
| *Salmonella* sp. | $3.7 \times 10^1$ | 1.63 | 0.92–2.08 | Berg and Berman, 1980; Lue-Hing et al., 1979; Jewell et al., 1980 |
| *Pseudomonas aeruginosa* | $6 \times 10^5$ | 0.58 | 0.15–1.36 | Berg and Berman, 1980; Lue-Hing et al., 1979; Jewell et al., 1980 |
| Enterovirus | $7.9 \times 10^1$ | 1.21 | 1.05–1.36 | Berg and Berman, 1980; Jewell et al., 1980 |

*Source*: Pedersen et al., 1981.

Proper mesophilic anaerobic digestion results in biosolids' meeting PSRP classification requirements or having a density of $<2 \times 10^6$ fecal coliform bacteria.

*Salmonella* sp. can survive mesophilic anaerobic digestion. Jones et al. (1983) reported that *Salmonella* sp. sampled during 12 days at two different treatment plants ranged from 3 to 24,000/100 ml in raw sewage and from 3 to 350/100 ml. On most days, there occurred several log reductions in organisms during 2 days in one plant. On 1 day in the second plant, the number of organisms was higher in the digested biosolids than in the raw sewage.

High rate mesophilic anaerobic digestion generally reduced pathogenic organisms and indicator bacteria by 1 to 2 logs as shown by a review of the literature prior to 1981 (see Table 7.11, Pedersen et al., 1981). New York City conducted one of the more comprehensive evaluations of pathogens and indicator organisms. Table 7.12 shows levels of pathogens and indicator organisms in anaerobically digested biosolids (NYCDEP, 1992). The biosolids were anaerobically digested for 20 days at 35°C. The data from New York showed that many pathogens and indicator organisms survived anaerobic digestion.

Soares et al. (1994) monitored enteroviruses and *Giardia* cysts in mesophilic anaerobically digested biosolids over a 14-month period. Enteroviruses ranged from $4.36 \times 10^3$ to $7.00 \times 10^5$ MPN/kg before anaerobic digestion and from 6.25 to $2.52 \times 10^5$ MPN/kg after.

A study at three small wastewater treatment plants in British Columbia evaluated the efficiency of ATAD. At 60 to 70°C and a hydraulic retention time of about 10 days, fecal coliform and fecal streptococcus, in seven of 12 samplings, were reduced

©2003 CRC Press LLC

**Table 7.12 Pathogens and Indicator Organisms in Mesophilic Anaerobically Digested Biosolids***

| Organism | Round 1 Sampling No./100 ml | Round 2 Sampling No./100 ml |
|---|---|---|
| Total coliform (plate count) | 110,000–9,900,000 | 14,000–19,000,000 |
| Fecal coliform (plate count) | 11,000–620,000 | 1,100–4,800,000 |
| Fecal coliform (plate count) | 1,100–350,000 | 1,100–2,400,000 |
| Fecal streptococci | 1,100–650,000 | 1,100–1,300,000 |
| Enterococci (membrane filtration) | 2,100–590,000 | 1,100–1,200,000 |
| *Clostridium perfringens* | 1,100–8,500,000 | 2,100,000–34,000,000 |
| *Salmonella* | 0.08–30 | 0.061–3 |
| *E. coli* C. | 5,000–890,000 | 500–30,000,000 |
| *Giardia lamblia* | 0.0–120 | 20–80 |
| *Ascaris lumbricoides* | 0.0–67 | 25–100 |
| *Ancylostoma necator* | 0.0–33 | Not recovered |
| *Enterobius vermicularis* | 0.0–33 | Not recovered |
| *Trichuris trichiura* | 0.0–33 | 25–100 |
| Total parasites | 13–170 | 6.2–280 |

*Source*: NYCDEP, 1992.

to less than 100 MPN/gram. Five samples had fecal streptococcus at or above 100 MPN/g. No salmonellae were detected (Kelly, 1991).

Cram (1943) found viable *Ascaris* eggs after 6 months of anaerobic digestion at mesophilic temperatures of 20–30 °C. Early studies at the University of Illinois showed that the ova of the round worm, *Ascaris l. suum*, survived the anaerobic digestion process and subsequently embryonated. The sludge digestion process protected the ova from temperatures of 38°C. When the ova were subsequently exposed to air, they embryonated. Stern and Farrell (1977) indicated that *Ascaris* ova survived thermophilic digestion at 50°C. Stadterman et al. (1995) reported that anaerobic digestion at 37°C inactivated 50% of *Cryptosporidium oocysts* after 2 hours of digestion, and after 24 h 99.98% were inactivated. However, Whitmore and Robertson (1995) indicated that mesophilic anaerobic digestion at temperatures ranging from 35°C to 37°C is incapable of destroying all oocysts.

Table 7.13 shows data on enteric virus concentration in anaerobically digested biosolids. Viruses ranged from <0.03 to 210 PFU.

**Table 7.13 Inactivation of Poliovirus in Composted Biosolids at 60% Moisture**

| Treatment | Percentage Recovery of Plaque-Forming Units |
|---|---|
| 35°C, 200 min | 30 |
| 39°C, 20 min | 7.2 |
| 43°C, 20 min | 0.087 |
| 47°C, 5 min | 0.003 |

©2003 CRC Press LLC

## Composting

The effect of composting on pathogen destruction is discussed in detail in the book *The Science of Composting* (Epstein, 1997). The composting process is capable of disinfecting wastes. However, either due to poor design or poor operations, the composting process enables some pathogens to survive (Yanko, 1988). The destruction of poliovirus in 40% compost solids, as related to temperature and treatment time, is shown in Table 7.13. At 35°C, poliovirus survived for a much longer period than at 47°C. Data on heat inactivation of total coliforms, fecal coliforms, fecal streptococcus and *Salmonella enteritidis* serotype Montevideo also showed substantial reduction of organisms at temperatures of 55°C to 65°C.

Knoll (1961) also described several experiments where he subjected different salmonella strains to composting temperatures at the Baden-Baden Biosolids-refuse composting plant. After 14 days of reactor time with temperatures of 55 °C to 60°C and a moisture content of 40% to 60%, the product did not contain pathogens.

Wiley (1962) reviewed some of the early literature on pathogen destruction by composting. He reported that pathogen destruction during composting is the result of thermal kill and antibiotic action, or by the decomposing organisms or their products. Knoll (1961) tested the theory that, besides temperature, antibiotic substances resulted in pathogen destruction by composting. He extracted a solution from composted material at different stages of the process. In a compost extract taken between days 7 and 16, no inoculated *Salmonella cairo* were able to grow. He determined that the development of inhibitors originated in the presence of actinomycetes and molds and concluded that this phenomena is due to an unknown antibiotic-producing organism. Although the optimum temperature that produces the antibiotic was not determined, he postulated that 50°C to 55°C appeared to be the temperature at which these substances were generated. Gaby (1975) did not find any oppressive or antagonistic material in compost. Golueke (1983) pointed out that indigenous organisms are in a better position to compete for nutrients than pathogenic microorganisms. Furthermore, he indicated that time acts as a factor since thermal destruction is not instantaneous. Time provides for the combination of several inhibitory factors to act on pathogenic organisms.

In 1969, Morgan and Macdonald investigated the fate of *Mycobacterium tuberculosis* during open windrow composting of biosolids and refuse at the U.S. Public Health Service–Tennessee Valley Authority research and demonstration compost plant in Johnson City, Tennessee. They found that the organism was destroyed after 10 days of composting when the temperature averaged 60°C. They also discovered that if the temperature remained low, the bacteria survived for long periods of time (until the temperature exceeded 44°C for extensive periods). They also pointed out that equipment used for handling compost that was less than 17 days old should not be used with finished compost to prevent reintroduction of pathogens into the finished product.

Gaby (1975) reported on a series of studies on pathogen reduction during windrowing of refuse-biosolids composting. *Salmonella* and *Shigella*, either originally present or introduced into refuse-biosolids mixtures, were not found within 7 to 21 days. Enteroviruses were not found in the raw refuse, biosolids, or a mixture of the

©2003 CRC Press LLC

two materials. Poliovirus Type 2 was introduced into the windrows, but was inactivated after 3 to 7 days. Human parasitic cysts and ova were introduced into the center of the windrow, but they disintegrated after 7 days. Dog parasitic ova did, however, survive for 35 days. *Leptospira philadelphia*, a spirochaete, did not survive for more than two days after introduction into the windrow.

Krogstad and Gudding (1975) inoculated solid waste and biosolids with *Salmonella typhimurium*, *Serratia marcescens* and *Bacillus cereus*. Periodic measurements were made to determine the die-off rate. The organism could not be detected after 4 days when the temperature in a horizontal drum composter was maintained at around 65°C. They concluded that 3 to 5 days in a reactor vessel with temperatures of 60°C to 65°C would destroy the pathogens studied. Walke (1975) monitored *Escherichia coli*, *Salmonella eidleberg* and *Candida albicans* during windrow composting of bark-biosolid mixtures. The initial compost contained these organisms at a level of $10^6$ microbes per dry gram. After 24 hours, the levels were 11, 130 and 620 microbes per dry gram of solids for *E. coli*, *Salmonella* sp. and *Candida albicans*, respectively. No organisms were detected after 36 h.

From 1973 to 1978, the U.S. Department of Agriculture conducted numerous studies on pathogen survival during composting by both the windrow and aerated static pile methods (Burge et al., 1978; Burge and Cramer, 1974; Epstein et al., 1977). The studies showed that salmonellae increased in growth initially, but was destroyed within 10 days of composting in the static pile and within 15 days in the windrow method. The destruction of an indicator virus, F2 bacteriophage, took 45 to 70 days in the windrow method and approximately 13 days in the aerated static pile method. This indicator virus was selected because it was more resistant to inactivation by heat than enteric pathogens, including viruses, bacteria, protozoa cysts and helminth ova.

Pathogenic microbial antagonism has been studied by several researchers (Brandon and Neuhauser, 1978; Millner et al., 1987). Millner et al. (1987) found that the types and numbers of different organisms affected the growth of salmonellae organisms. The presence of coliforms only or metabolically active bacteria and actinomycetes resulted in the death of salmonellae in compost.

During composting, three factors can result in the destruction of pathogens:

1. Time–temperature
2. Production of ammonia
3. Presence of competing organisms

Some concern for regrowth of pathogens exists, even when high temperatures have been achieved. Organisms could move from cooler sections of piles or windrows into areas that have previously been hot enough to eliminate pathogens. It is important to ensure uniform high temperatures in piles and windrows. Furthermore, by achieving stabilization, the food source is diminished and pathogens cannot compete with indigenous microbial populations for the remaining food. Burge et al. (1978) has shown evidence of this and also found that additional die-off of pathogens occurred during curing.

©2003 CRC Press LLC

### Heat Drying

Heat drying should completely destroy pathogens. Farrell and Stern (1975) rated heat treatment at 195°C as an excellent method of pathogen destruction. Heat treatment was rated poor for putrefaction potential and odor attenuation. Since antagonistic or competitive microorganisms are destroyed, contamination of the product can result in the growth of a pathogen to very high levels (Ward and Brandon, 1977; Brandon et al., 1977).

### Alkaline Stabilization

Lime treatment of biosolids was recognized early as a method of deodorizing and disinfecting the material. USEPA 503 regulations require that the pH of biosolids be increased to 12.0 for a minimum of 2 hours. Because ammonia is released during the addition of lime, this compound could act as a disinfectant. If the addition of lime is insufficient to maintain the pH for the time required to disinfect the biosolids, the pH will drop and surviving bacteria will grow when conditions become favorable.

Pedersen et al. (1981) indicated that lime stabilization can result in 1 to 7 log reductions in some indicator and pathogenic organisms. Sepp (1980) concluded that helminth ova remained viable for long periods of time even when the pH of biosolids was at 12. Counts and Shuckrow (1974) found a 7-log reduction in fecal coliform at a pH of 12.4 in 2% biosolids. Lower values were obtained for 4.4% solids. For *Salmonella* spp., approximately 1 to 2 log reductions occurred. Burnham (1986) reported that, within 5 weeks, 25% and 35% cement kiln dust (CKD) and lime were effective in meeting PFRP (Class A Biosolid) and resulted in the destruction of *Salmonella*. Enterovirus levels were controlled to PFRP levels within 1 day by CKD and lime treatment. *Ascaris* egg survival was reduced by more than 3 log at high CKD and lime treatment within 4 weeks.

## CONCLUSION

The data show that wastewater treatment systems generally were very effective in reducing the levels of pathogens. USEPA (1999) summarized the effect of biosolids treatment on pathogens (Table 7.14). Further treatment of the solids results in disinfection and destruction of pathogens. Biosolid treatments that achieve a Class A level as defined by USEPA 40 CFR 503 are very effective in eliminating pathogens. USEPA has determined that for Class B biosolids that could harbor pathogens, additional site restrictions are needed to prevent contamination of food, feed and water resources.

The potential for entry of pathogens into the food chain is the ultimate concern of humans and animals. This depends on the survival of pathogens in soil and plants. Furthermore, crop management practices play an important role in preventing contamination and ensuring quality of food and feed products. The next chapter deals with the survival of pathogens in soils and on crops and discusses contamination of air and water resources.

©2003 CRC Press LLC

**Table 7.14 Effects of Biosolids Treatment on Pathogens**[a]

| PFRP Treatment | Bacteria | Viruses | Parasites (Protozoa and Helminths) |
|---|---|---|---|
| Anaerobic digestion | 0.5–4.0 | 0.5–2.0 | 0.5 |
| Aerobic digestion | 0.5–4.0 | 0.5–2.0 | 0.5 |
| Composting (PFRP) | 2.0–4.0 | 2.0–4.0 | 2.0–4.0 |
| Air drying | 0.5–4.0 | 0.5–4.0 | 0.5–4.0 |
| Lime stabilization | 0.5–4.0 | 4.0 | 0.5 |

[a]Log reductions shown: A 1-log reduction (tenfold) is equal to a 90% reduction. Class B processes are based on a 2-log reduction.

## REFERENCES


Akin, E.W., 1983, Infective dose of waterborne pathogens, Proc. 2nd National Symposium Municipal Wastewater Disinfection, Orlando, Florida, Health Effects Research Laboratory, Cincinnati, OH.

Anonymous, 1975, New chlorine application improves viral kill, *Water Sewage Works*, 30: R-68.

Berg, G. and D. Berman, 1980, Destruction by anaerobic mesophilic and thermophilic digestion of viruses and indicator bacteria indigenous to domestic sludges, *Appl. Environ. Microb.* 39: 361–368.

Besser, R.E., S.M. Lett, J.T. Weber, M.P. Doyle, T.J. Barrett, J.G. Wells and P.M. Griffin, 1993, An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* 0157:H7 in fresh-pressed apple cider, *JAMA* 269: 2217.

Bovay Engineers, Inc., 1975, Feasibility of land application for Spokane, Washington wastewater solids, Spokane, WA.

Brandon, J.R. and K.S. Neuhauser, 1978, Moisture effects on inactivation and growth of bacterial and fungi in sludges, Sandia Laboratories, Publ. SAND 78–1304, Albuquerque.

Brandon, J.R., W.D. Burge and N.E. Enkiri, 1977, Inactivation by ionizing radiation of *Salmonella enteritidis* serotype Montevideo growth in composted sewage sludge, *Appl. Environ. Microbiol.* 33: 1011–1012.

Bryan, F.L., 1977, Disease transmitted by foods contaminated by wastewater, *J. Food Protection* 40: 45–52.

Burge, W.D. and W.N. Cramer, 1974, Destruction of pathogens by composting sewage sludge, USDA Agricultural Research Service and Maryland Environmental Service and Water Resources Management, Beltsville, MD.

Burge, W.D. and P.D. Millner, 1980, Health aspects of composting: Primary and secondary pathogens, pp. 245–266, G. Bitton, B.L. Damron, G.T. Edds and J.M. Davidson (Eds.), *Sludge — Health Risks of Land Application*, Ann Arbor Science, Ann Arbor, MI.

Burge, W.D., W.N. Cramer and E. Epstein, 1978, Destruction of pathogens in sewage sludge by composting, *Trans. ASAE* 21: 510–514.

Burnham, J.C., 1986, The effect of cement kiln dust and lime on microbial survival in Toledo municipal wastewater sludges, Report from the Department of Microbiology, Medical College of Ohio, Toledo.

Casmore, D.P., 1991, The epidemiology of human cryptosporidiosis and the water route of infection, *Water Sci. Techol*. 24: 157–164.


©2003 CRC Press LLC

Casson, L.W., M.O.D. Ritter, L.M. Cossentino and P. Gupta, 1997, Survival and recovery of seeded HIV in water and wastewater, *Water Environ. Res*. 69: 174–179.

Casson, L.W., C.A. Sorber, R.H. Palmer, A. Enrico and P. Gupta, 1992, HIV survivability in wastewater, *Water Environ Res*. 64: 213.

Cave, D.R., 1997, Epidemiology and transmission of *Helicobacter pylori* infection, *Gastroenterology* 113: S9–S14.

Clarke, N., R.E. Stevenson, S.L. Chang and P.W. Kabler, 1961, Removal of enteric viruses from sewage by activated sludge, *Am. J. Public Health* 51: 1118.

Clarke, N., G. Berg, P.W. Kabler and S.L. Chang, 1964, Human enteric viruses in Water: Source, survival and removability, *Advances in Water Pollution Research–Proc. Int. Conf*., London, England, Pergamon, London.

Clarke, N. and S. Chang, 1975, Removal of enteroviruses from sewage by bench-scale rotary-tube trickling filters, *Appl. Microbiol*. 30: 233.

Cliver, D.O., 1975, Virus association with wastewater solids, *Environ. Lett.* 10: 215–223.

Counts, C.A. and A.J. Shuckrow, 1974, Lime stabilized sludge — its stability and effect on agricultural land, U.S. Environmental Protection Agency, Nat. Environ. Res. Center, Cincinnati, OH.

Cram, E.B., 1943, The effect of various treatment processes on the survival of helminth ova and protozoan cysts in sewage, *Sewage Works*, 15: 1119–1138.

Dahab, M.F., R. Surampalli and P. Ponugoti, 1996, Pathogen and pathogen indicator reduction characteristics in municipal biosolids treatment systems, pp. 265–276, WEFTEC ’96, 69th Annual Conference and Exposition, Part I: Residuals and Biosolids Management, Dallas.

Dean, R.S. and J.E. Smith, 1973, The properties of sludge, pp. 39–47, *Proc. Joint Conference on Recycling Municipal Sludges and Effluents on Land*, National Association of State Land-Grant Colleges, Washington, D.C.

Dupont, H.L., C.L. Chappell, C.R. Sterling, P.C. Okhuysen, J.B. Rose and W. Jakubowski, 1995, The infectivity of *Cryptosporidium parvum* in healthy volunteers, *N. Engl. J. Med.* 332: 855–859.

Engineering Science, 1987, Monterey wastewater reclamation study for agriculture, Final Report, Engineering Science, April 1987, Berkeley, CA.

England, B., R.E. Leach, B. Adams and R. Shiosak, 1967, Virologic assessment of sewage treatment at Santee, California, p. 401, in G. Berg (Ed.), *Transmission of Viruses by Water Route*, Interscience, New York.

Enriquez, C.E., C.P. Gerba and M. Abbaszadegan, 1993, Survival of human immunodeficiency virus (HIV) in water and wastewater, p. 859, *Proc. 1993 Water Quality Technol. Conf. Am. Water Works Assoc*.

Epstein, E., 1997, *The Science of Composting*, Technomic, Lancaster, PA.

Epstein, E. and J.F. Donovan, 1992, Pathogens in composting and their fate, *Proc. of Conf. Pathogens in Sludge: What Does it Mean?*, New Orleans, Water Environment Federation.

Epstein, E., J.F. Parr and W.D. Burge, 1977, Health aspects of land application of sewage sludge and sludge compost, National Conference Hazardous Waste Management, San Francisco, Information Transfer, Inc., Rockville, MD.

Farrah, S.R., G. Bitton, E.M. Hoffman, O. Lanni, O.C. Pancorbo, M.C. Lutrick and J.E. Bertrand, 1981, Survival of entericviruses and coliform bacteria in a sludge lagoon, *Appl. Environ. Microbiol*. 41: 459–465.

Farrell, J.B., 1992, Technical support document for reduction of pathogens and vector attraction in sewage sludge, U.S. Environmental Protection Agency, EPA 822/R-93-004, Washington, D.C.

©2003 CRC Press LLC

Farrell, J.B. and G. Stern, 1975, Methods for reducing the infection hazard of wastewater sludge, pp. 19–28, *Radiation for a Clean Environment, Proc. Int. Symp. on the Use of High Level Radiation in Waste Treatment — Status and Prospects*, Munich, Germany.

Farrell, J.B., V. Bhide and J.E. Smith, Jr., 1996, Development of EPA's new methods to quantify vector attraction of wastewater sludges, *Water Environ. Res.* 68: 286–294.

Feachem, R.G., D.J. Bradley, H. Garelick and D.D. Mara, 1980, Appropriate technology for water supply and sanitation: Health effects of excreta and silage management — a state of the art review, World Bank, Washington, D.C.

Fitzgerald, P.R. and R.F. Ashley, 1977, Differential survival of Ascaris ova in wastewater sludge, *J. Water Pollut. Control Fed.* 49: 1722–1724.

Foster, D.H. and R.S. Engelbrecht, 1973, Microbial hazards in disposing of wastewater on soil, pp. 247–270, W.E. Sopper and L.T. Kardos (Eds.), *Recycling Treated Municipal Wastewater and Sludge through Forest and Cropland*, Pennsylvania State University Press, University Park.

Fradkin, L., S.M. Goyal, R.J.F. Bruins, C.P. Gerba, P. Scarpino and J.F. Stara, 1989, Municipal wastewater sludge, The potential public health impacts of common pathogens, *J. Environ. Health* 51: 148–152.

Gaby, W.L., 1975, Evaluation of health hazards associated with solid waste/sewage sludge mixtures, USEPA National Environ. Res. Center, Office of Res. and Dev. EPA-670/2-75-023, Cincinnati, OH.

Gerba, C.P., 1983, Pathogens, pp. 147–195, A.L. Page, T.L. Gleason, J.E. Smith, I.K. Iskander and L.E. Sommers (Eds.), *Utilization of Municipal Wastewater and Sludge on Land*, University of California, Riverside.

Gilbert, J.V., J. Ramakrishna, F.W. Sunderman, Jr., A. Wright and A.G. Plaut, 1995, Protein Hpn: Cloning and characteristics of a histidine-rich metal binding polypeptide in *Helicobacter pylori* and *Heliobacter mustelae, Infect. Immun.* 63: 2682–2688.

Golueke, C.G., 1983, Epidemiological aspects of sludge handling and management, Part II. *BioCycle* 24: 52–58.

Grabow, W.O.K, 1968, The virology of waste water treatment, *Water Res.* 2: 675.

Grubel, P., J.S. Hoffman, F.K. Chong, N.A. Burstein, C. Mepani and D.R. Cave, 1997, Vector potential of houseflies (*Musca domestica*) for *Helicobacter pylori*, *J. Clin. Microbiol.*

Hornick, R.B., S.E. Greisman, T. E. Woodward, H.L. Dupont, A.T. Dawkins and M.J. Snyder, 1970, Typhoid fever: Pathogenesis and immunological control, *New Engl. J. Med*. 283: 686–691.

Hurst, C., S. Farrah, C. Gerba and J. Melnick, 1978, Development of quantitative methods for the detection of enteroviruses in sewage sludges during activation and following land disposal, *Appl. Environ. Microbiol*. 36: 81–89.

Jewell, W.J., R.M. Kabrick and J.A. Spada, 1980, Autoheated aerobic thermophilic digestion with air aeration, Municipal Research Lab., U.S. Environmental Protection Agency, R804636, Cincinnati, OH.

Johnson, R.W., E.R. Blatchley III and D.R. Mason, 1994, HIV and the bloodborne pathogen regulation: Implications for the wastewater industry, *Water Environ. Res.* 66: 684–688.

Jones, F., A.F. Godfree, P. Rhodes and D.C. Watson, 1983, Salmonellae and sewage sludge — Microbiological monitoring, standards and control in disposing sludge to agricultural lands, pp. 95–114, P.M. Wallis and D.L. Lehmann (Eds.), *Biological Health Risks of Sludge Disposal to Land in Cold Regions*, University of Calgary Press, Alberta.

Kebina, V.Y. and G.L. Ploshcheva, 1974, Sanitary helminthological evaluation of the waste water treatment method on a small industrial sewage plant, *Gig. Sanit*. 7: 93.

©2003 CRC Press LLC

Keller, J., 1974, Ozone disinfection pilot plant studies at Laconia, New Hampshire, *J. Am. Water Works Assoc*. 66: 734.

Kelly, H.G. (Ed.), 1991, Autothermal thermophilic aerobic digestion of municipal sludges: Conclusions of a 1-year full scale demonstration project, 64th Annual Conf. of the Water Pollution Control Fed. Toronto.

Kelly, S.M., W.W. Sanderson and C. Neidl, 1961, Removal of enteroviruses from sewage by activated sludge, *J. Water Pollut. Control Fed*. 33: 1056.

Keswick, B.H., 1984, Sources of groundwater pollution, pp. 39–64, G. Bitton and G. Gerba (Eds.), *Groundwater Pollution Microbiology*, John Wiley & Sons, New York.

Knoll, K.H., 1961, Public health and refuse disposal, *Compost Sci*. 2: 35–40.

Koenraad, P.M.F.J., W.F. Jacobs-Reitsma, R.R. Breumer and F.M. Rombouts, 1996, *Campylobacter* spp. in a sewage plant and in the waste water of a connected poultry slaughterhouse, *Water Environ. Res*. 188.

Koenraad, P.M.FJ., F.M. Rombouts and S.H.W. Notermans, 1997, Epidemiological aspects of thermophilic *Campylobacter* in water-related environments: A review, *Water Environ. Res*. 69: 52–63.

Kott, Y., E.M. Nupen and W.R. Ross, 1975, The effect of pH on the efficiency of chlorine disinfection and virus enumeration, *Water Res*. 9: 869.

Kowal, N.E., 1983, An overview of public health effects, pp. 329–394, A.L. Page, T.L. Gleason, J.E. Smith, I.K. Iskander and L.E. Sommers (Eds.), *Utilization of Municipal Wastewater and Sludge on Land*, University of California, Riverside.

Kowal, N.E., 1985, Health effects of land application of municipal sludge, U.S. Environmental Protection Agency, Health Effects Res. Lab., Rep. No. EPA 600/1-85-015, Research Triangle Park, NC.

Krogstad, O. and R. Gudding, 1975, The survival of some pathogenic microorganisms during reactor composting, *Acta Agric. Scandinavia* 25: 281–284.

Laconde, K., R. Lofy and R. Stearns, 1978a, Municipal sludge agricultural utilization practices: An environmental assessment, Vol. I. U.S. Environmental Protection Agency, EPA 530-SW-709, Cincinnati, OH.

Laconde, K., R. Lofy and R. Stearns, 1978b, Municipal sludge agricultural utilization practices, Vol. II, U.S. Environmental Protection Agency, EPA/530/SW-156C, Cincinnati, OH.

Levine, M.M., H.L. Dupont and S.B. Formal, 1973, Pathogenesis of *Shigella dysenteriae* (Shiga) dysentery, *J. Infect. Dis*. 127: 261–270.

Lue-Hing, C., S.J. Sedita and K.C. Rao, 1979, Viral and bacterial levels resulting from land application of digested sludge, pp. 445–462, W.E. Sopper and S.N. Kerr (Eds.), *Utilization of Municipal Sewage Effluent and Sludge on Forest and Disturbed Land*, University Press, College Park, PA.

Majumdar, S.B., W.H. Ceckler and O.J. Sproul, 1974, Inactivation of polio virus in water by ozonation, *J. Water Pollut. Control Fed*. 46: 2048.

Malina, J.F., Jr., 1976, Viral pathogen inactivation during treatment of municipal wastewater, pp. 9–23, L.B. Baldwin, J.M. Davidson and J.F. Gerber (Eds.), *Virus Aspects of Applying Municipal Wastes to Land*, Center for Environmental Programs, Institute of Food and Agricultural Sciences, University of Florida, Gainesville.

Malina, J.F., Jr. and A. Melbard, 1974, Inactivation of virus in bench-scale oxygenated waste stabilization ponds, University of Texas, Austin.

Malina, J.F., Jr., K. Ranganathan, B.P. Sagik and B.E. Moore, 1975, Polio inactivation by activated sludge, *J. Water Pollut. Control Fed*. 47: 2178.

©2003 CRC Press LLC

Millner, P.D., K.E. Powers, N.K. Enkiri and W.D. Burge, 1987, Microbial mediated growth suppression and death of salmonella in composted sewage sludge, *Microb. Ecol*. 14: 255–265.

Moore, B.E., B.P. Sagic and C.A. Sorber, 1978, Land application of sludges: Minimizing the impact of viruses on water resources, *Proc. Conf. on Risk Assessment and Health Effects of Land Application of Municipal Wastewater and Sludges*, San Antonio, TX.

Morgan, M.T. and F.W. Macdonald, 1969, Tests show MB tuberculosis doesn't survive composting, *J. Environ. Health* 32: 101–108.

Nath, M.W. and J.C. Johnson, 1979, Quantitative enumeration and evaporation-induced inactivation of enteric viruses in wastewater sludge, Virginia Water Resources Research Center, Virginia Polytechnic Institute, Blacksburg.

Nielsen, A.L. and B. Lydholm, 1980, Methods for the isolation of virus and raw and digested wastewater sludge, *Water Res*. 14: 175–178.

Noland, R.F., J.D. Edwards and M. Kipp, 1978, Full scale demonstration of lime stabilization, U.S. Environmental Protection Agency, EPA-600/2-78-171, Cincinnati, OH.

Novak, J.T., M.P. Eichelberger, S.K. Banerji and J. Yaun, 1984, Stabilization of sludge from an oxidation ditch, *J. Water Pollut. Control Fed.* 56: 950–954.

NRC, 1996, Use of reclaimed water and sludge in food crop production, National Research Council, 1996, Washington, D.C.

NYCDEP, 1992, Sludge management plan, generic environmental impact statement, Chapter IV, Public Health, New York City Department of Environmental Protection, New York.

Pahren, H.R., 1987, Microorganisms in municipal solid waste and public health implications, *CRC Crit. Rev. Environ. Control* 17(3): 187–228.

Parsons, H.R., C. Brownlee, D. Wetter, A. Maurer, E. Haughton, L. Kordner and M. Slezak, 1975, Health aspects of sewage effluent irrigation, Pollution Control Branch, British Columbia Water Resources Service, Victoria.

Pavoni, J. and M. Tittlebaum, 1974, Virus inactivation in secondary wastewater treatment plant effluent using ozone, p. 189, J.F. Malina, Jr. and B.P. Sagik (Eds.), *Virus Survival in Water and Wastewater Systems*, Center for Research in Water Resources, University of Texas, Austin.

Pedersen, D.C., 1981, Density levels of pathogenic organisms in municipal wastewater sludge — a literature review, U.S. Environmental Protection Agency, EPA-600/S2-81-170, Cincinnati, OH.

Pedersen, D.C., A.B. Pincince and J.E. Bates, 1981, Reduction of bacteria, viruses and parasites during conventional sludge treatment processes, Water Pollution Control Federation 54th Annual Conference, Detroit, MI.

Pell, N., 1997, Manures and microbes: Public health and animal health problem? *J. Dairy Sci*. 89: 2673–2681.

Ranganathan, K., J.F. Malina, Jr., and B.P. Sagik (Eds.), 1974, Inactivation of enteric virus during biological wastewater treatment, 7th International Conference on Water Pollution Research, Progress in Water Technology, Pergamon, London.

Reimers, R.S., M.D. Little, D.B. Leftwich, D.D. Bowman, E.J.J. Englande and R.F. Wilkenson, 1980, Parasites in southern sludges and disinfection by standard sludge treatment, National Technical Information Service, EPA Pub. 600/2-81-166. NTIS No. PB82 102344, Springfield, VA.

Reimers, R.S., W.S. Bankston, G.L. Goldstein, Y. Yang and S. Liu, 1996, Disinfection of pathogens by biosolids processing, pp. 51–74, *Stabilization and Disinfection — What Are Our Concerns?*, Water Environment Federation, Dallas, TX.

SAC, 1979, Sewage sludge management program, Vol. 6, Microbiological and virus studies, Sacramento Area Consultants, California.

©2003 CRC Press LLC

Sack, R.B., 1975, Human diarrheal disease caused by enterotoxigenic *Escherichia coli*, *Annu. Rev. Microbiol.* 29: 333–353.

Sekla, L., D. Gemmill, J. Manfreda, M. Lysyk, W. Stackiw, C. Kay, C. Hopper, L. VanBuckenhouat and G. Eibisch, 1980, Sewage treatment plant workers and their environment: A health study, U.S. Environmental Protection Agency, EPA-600/9-80-028, Cincinnati, OH.

Sepp, E., 1980, Pathogen survival in sludge stabilization processes, California Dept. of Health Services, Sacramento.

Sherman, V., 1975, Virus removal in trickling filter plants, *Water and Sewage Works*, April 30: R-36.

Slade, J.S., E.B. Pike, R.P. Eglin, J.S. Colbourne and J.B. Kurtz, 1989, The survival of human immunodeficiency virus in water, sewage and sea water, *Water Sci. Technol*. 21: 55.

Smith, J.E. and J.B. Farrell, 1996, Current and future disinfection — federal perspectives, Water Environ. Fed. 69th Annual Conf. and Exposition, Workshop on Stabilization and Disinfection — What Are Our Concerns? October 5, 1996, Chicago, IL.

Soares, A.C., T.M. Straub, I.L. Petter and C.P. Gerba, 1994, Effect of anaerobic digestion on the occurrence of enteroviruses and giardia cysts in sewage sludge, *J. Environ. Sci. Health A, Environ. Sci. Eng.* 29: 1887–1897.

Sorber, C., B. Moore, D. Johnson, H. Harding and R. Thomas, 1984, Microbial aerosols from application of liquid sludge to land, *J. Water Pollut. Control Fed.* 56: 830.

Stadterman, K.L., A.M. Sninsky, J.L. Sykora and W. Jakubowski, 1995, Removal and inactivation of *Cryptosporidium* oocysts by activated sludge treatment and anaerobic digestion, *Water Sci. Technol*. 31: 97–104.

*Stedman's Medical Dictionary*, 1977, 23rd ed., Williams & Wilkins, Baltimore, MD.

Stern, G. and J.B. Farrell (Eds.), 1977, Sludge disinfection techniques. *Proc. Nat'l Conf. on Composting of Municipal Residues and Sludges*, Information Transfer, Rockville, MD.

Theis, J.H., V. Bolton and D.R. Storm, 1978, Helminth ova in soil and sludge from twelve U.S. urban areas, *J. Water Pollut. Control Fed.* 50: 2485–2493.

USEPA, 1991, Preliminary Risk Assessment for Parasites in Municipal Sewage Sludge Applied to Land, U.S. Environmental Protection Agency, EPA 600/6-91/001, Washington, D.C.

USEPA, 1992, Technical Support Document for Reduction of Pathogens and Vector Attraction in Sewage Sludge, U.S. Environmental Protection Agency, EPA R-93-004, Washington, D.C.

USEPA, 1999, Control of Pathogens and Vector Attraction in Sewage Sludge, U.S. Environmental Protection Agency, Office of Research and Development, National Risk Management Research Laboratory, Center for Environmental Research Information, EPA/625/R-92-013, Cincinnati, OH.

Vik, T.E. and J.R. Kirk, 1996, Operating experience with the nation's first and world's largest auto-thermal aerobic digestion system, 10th Annual Residual and Biosolids Management Conference: 10 Years of Progress and a Look Toward the Future, Water Environment Federation, Denver, CO.

Walke, R., 1975, The preparation, characterization and agricultural use of bark-sewage compost.

Wang, G., T. Zhao and M.P. Doyle, 1996, Fate of enterohemorrhagic *Escherichia coli* 0157:H7 in bovine feces, *Appl. Environ. Microbiol.* 62: 2567.

Ward, R.L. and J.R. Brandon, (Ed.), 1977, Effect of heat on pathogenic organisms found in wastewater sludge, pp. 122–134, Nat'l. Conf. on Composting Municipal Residues and Sludges, Information Transfer, Rockville, MD.

©2003 CRC Press LLC

Ward, R.L., G.A. McFeters and J.G. Yeager, 1984, Pathogens in sludge: Occurrence, inactivation and potential regrowth, Sandia National Laboratory, Sand 83–0557.TTC-0428, Albuquerque.

Ward, R.L., D.I. Berstein, E.C. Young, J.R. Sherwood, D.R. Knowlton and G.M. Schiff, 1986, Human rotavirus studies in volunteers: Determination of infectious dose and serological response to infection, *J. Infect. Dis*. 154: 871–880.

Wellings, F.M., A.L. Lewis and C.W. Mountain, 1976, Demonstration of solids-associated virus in wastewater and sludge, *Appl. Environ. Microbiol*. 31: 354–358.

Whitmore, T.N. and L.J. Robertson, 1995, The effect of sewage sludge treatment process on oocysts of *Cryptosporidium parvum*, *J. Appl. Microbiol*. 78: 34–38.

Wiley, J.S., 1962, Pathogen survival in composting municipal wastes, *J. Water Pollut. Control Fed*. 34: 80–90.

Yanko, W.A., 1988, Occurrence of pathogens in distribution and marketing municipal sludges, County Sanitation Districts of Los Angeles County, San Jose Creek Water Quality Laboratory, EPA/600/1-87/014, 1980, Cincinnati, OH.

Yanko, W.A., A.S. Walker, J.J. Jackson, L.L. Libao and A.L. Garcia, 1995, Enumerating salmonella in biosolids for compliance with pathogen regulations, *Water Environ. Res*. 67: 364–370.

©2003 CRC Press LLC